

# A study of intraspecific variation in the green tree frog *Litoria chloris* (Boulenger) (Hylidae)

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## ABSTRACT

The morphological and osteological variation of three disjunct populations of *Litoria chloris* have been subjected to a number of analyses. Interpopulation differences include size of adult, shape of the head and thigh colouration. It is conceivable that the species as now recognised includes more than one taxon. Biological data would confirm or refute a supposition derived from morphometric data.

## INTRODUCTION

The importance of a detailed knowledge of intraspecific variation amongst anuran populations as a prerequisite to meaningful interspecific comparisons has been appreciated only recently. Extensive gene flow within a population can produce such variation that the interpretation of character states used in systematic studies can be altered radically.

Trueb (1977) has shown considerable variation in osteological characters within a single population of the tree frog *Hyla lanciformis*, and extensive variations in morphological, osteological and behavioural characters have been shown over an altitudinal gradient in *Hyla lancasteri* (Trueb, 1968). Limited variation in cranial characters within three geographically disjunct populations of *Litoria infrafrenata* was found by Davies (1978).

There exist in Australia instances in which two or more disjunct frog populations exhibit variation in morphological characters, so leading to a degree of uncertainty regarding the taxonomic status of such populations. The hylid frog *Litoria chloris* (Boulenger) is such an example. It occurs in closed rainforests in

Queensland and New South Wales along a narrow coastal strip of some few kilometres wide which extends over one thousand kilometres. Disjunctions within this range are probably of recent origin being correlated with the destruction by European man of coastal rainforest.

Here we record morphological and osteological variation within *Litoria chloris*, together with previously unpublished data on natural history, and attempt to correlate such variation with complementary geographic isolation.

## MATERIALS AND METHODS

Abbreviations: A.M. Australian Museum; A.M.N.H. American Museum of Natural History; K.U. University of Kansas, Museum of Natural History; L.A.C.M. Los Angeles County Museum; N.P.W.S. Queensland National Parks & Wildlife Service; Q.M. Queensland Museum; S.A.M. South Australian Museum; U.A.Z. Zoology Department, University of Adelaide; N.P. National Park; S.F. State Forest. Letters following acronyms are departmental registration abbreviations.

Specimens examined: Specimens or series of specimens marked with an asterisk have not been included in the morphological analysis, but are distribution records.

*Northern Population:* Q.M. J.17109, Atherton; Q.M. J.17110, Crystal Cascades; Q.M. J. 27105-6, Little Forks; Q.M. J.25194-5\*, Q.M. J.25258-60, Home Rule Falls, Cooktown; Q.M. J.25278\*,  $\frac{1}{4}$  mile east of "Granite", Home Rule; N.P.W.S. A.252-3, Longmans Gap; N.P.W.S. A.255-7, Lake Barrine N.P.; N.P.W.S. Q.P.A.026-7, Severin, Boar Pocket; N.P.W.S. N.14292-3, Crater N.P.; N.P.W.S. N.14270-3, N.P.W.S. N.14274-5\*, N.P.W.S. N.14276, Palmerston N.P.; N.P.W.S. N.14310-1, Mt. Lewis S.F.; N.P.W.S. N.14162-3, McDowell Range; N.P.W.S. N.14185-6, Mt. Baldy, south-west Atherton; N.P.W.S. N.14298-9\*, Lake Eacham N.P.; N.P.W.S. N.28113-4\*, Gadgarra S.F.; N.P.W.S. N.14533-43\*, Charappa Creek; K.U. 147231\*, Tinaroo Creek Road, Mareeba; L.A.C.M. 41136-58\*, 50732\*, Lake Eacham, east of Atherton; L.A.C.M. 41163\*, 41165\*, 5-10 miles south-west of Cairns; L.A.C.M. 41173\*, 41182\*, 41193\*, 41195", Yungaburra; A.M.N.H. 19939\*, Vine Creek near Ravenshoe; A.M.N.H. 54176\*, Maalan Sutties Gap Road, 12 miles south of Millaa Millaa; S.A.M. R.16794-8, Gadgarra S.F.; U.A.Z. A.23, D.322, Gadgarra S.F.; Q.M. J.30902\*, J.32081\*, J.32140\*, Paluma, Mt. Spec.

*Central Population:* N.P.W.S. N.11819-20, Bulburin S.F.; N.P.W.S. A.882, Conway N.P.; N.P.W.S. A.914-8, Eungella N.P.; N.P.W.S. N.12295-7, N.12339-80, Eungella N.P.; Q.M. J.23847-8, Bulburin via Lowmead; S.A.M. R.16780-3, R.16828, Eungella N.P.; U.A.Z. A22, A.25, A.27, D323-7, Eungella N.P..

*Southern Population:* Q.M. J.28249\*, Cooyar-Maidenwell Road; Q.M. J.29009\*, Upper Reaches, Bulumba Creek, Kenilworth S.F.; Q.M. J.13119\*,

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Beechmont; Q.M. J.27545, J.27551, Bunya Mountains; Q.M.J.17102\*, J.17105\*, J.17107-8\*, Mt. Nebo; Q.M. J.17103, Methodist Youth Camp, Cunningham's Gap; Q.M. J.17104, J.17106, J.17099, Dum Dum-Murwilla Road; Q.M. J.17101, Dum Dum; Q.M. J. 23692, J.23694, Mt. Glorious; N.P.W.S. A.10, Mt. Tamborine; N.P.W.S. A.478\*, A.325, A.327, Warrie N.P.; N.P.W.S. A. 487, A.537, Bunya Mountains N.P.; N.P.W.S. A.576, Ravensbourne N.P.; N.P.W.S. A.586, Mt. Glorious near Maida N.P.; N.P.W.S. N.11821-2, Mudgereeba Road, near Springbrook; N.P.W.S. N.11775-7, Conondale Range, Bellthorpe S.F.; N.P.W.S. A.560-1, A.122-3, Lamington N.P.; N.P.W.S. N.17930\*, N.28268\*, Kilcoy Creek, Conondale Range; N.P.W.S. N.28280-2\*, Murumba Creek, Jimma S.F.; S.A.M. R.16786-93, Conondale Range, Bellthorpe S.F.; S.A.M. R.16784-5, Warrie N.P., Springbrook; A.M. R.5846, Woolongbar, Richmond River; A.M. R.7339, Goangara near Walgett, N.S.W.; A.M. R.7484-7, R. 7490-1, Dunoon, Richmond River, N.S.W.; A.M. R.9902, R.25992, Gosford, N.S.W.; A.M. R.1635-6, Lowana, N.S.W.; A.M. R.35535-42, R.35544-57, Boyd River on Oakwood Fire Trail; A.M.N.H. 62909-10\*, Dunoon, Richmond River; K.U. 138729\*, Tumbi Umbi, N.S.W.; U.A.Z. A.26, D328-33, Warrie N.P., Springbrook.

Specimens for skeletal examination were cleared and stained using Alizarin Red S following slight modification of the technique of Davis and Gore (1947). Modification included differing times in solutions, all end points being subjectively estimated. Dried skeletons were prepared by manual flensing of the majority of soft tissue, then immersing the bones in a dilute solution of sodium hypochlorite to remove the remaining tissue and bleach the bones. All skeletal material is registered in the University of Adelaide Zoology Department Collection (UAZ).

Osteological descriptions follow Trueb (1973) and morphological descriptions follow Tyler (1968). External morphological measurements were taken using dial calipers following the method of Tyler (1968). Measurements were recorded in millimetres. These were: snout to vent length (S-V); tibia length (TL); head length (HL); head width (HW); eye to naris distance (E-N); internarial span (IN); eye diameter (E); tympanum diameter (T). From these the following ratios were calculated: TL/S-V; E-N/IN; HL/HW; HL/S-V.

Data were analysed using a student t-test and results displayed graphically by the method of Simpson, Roe and Lewontin (1960) based on that of Hubbs and Hubbs (1953).

### GEOGRAPHICAL DISTRIBUTION

The distribution of *Litoria chloris* is shown in Figure 1. Three disjunct areas are apparent and these have been defined as follows:

1. Northern population; that area ranging from Cooktown to Mt. Spec.
2. Central population; that area ranging from Proserpine to Bulburin.

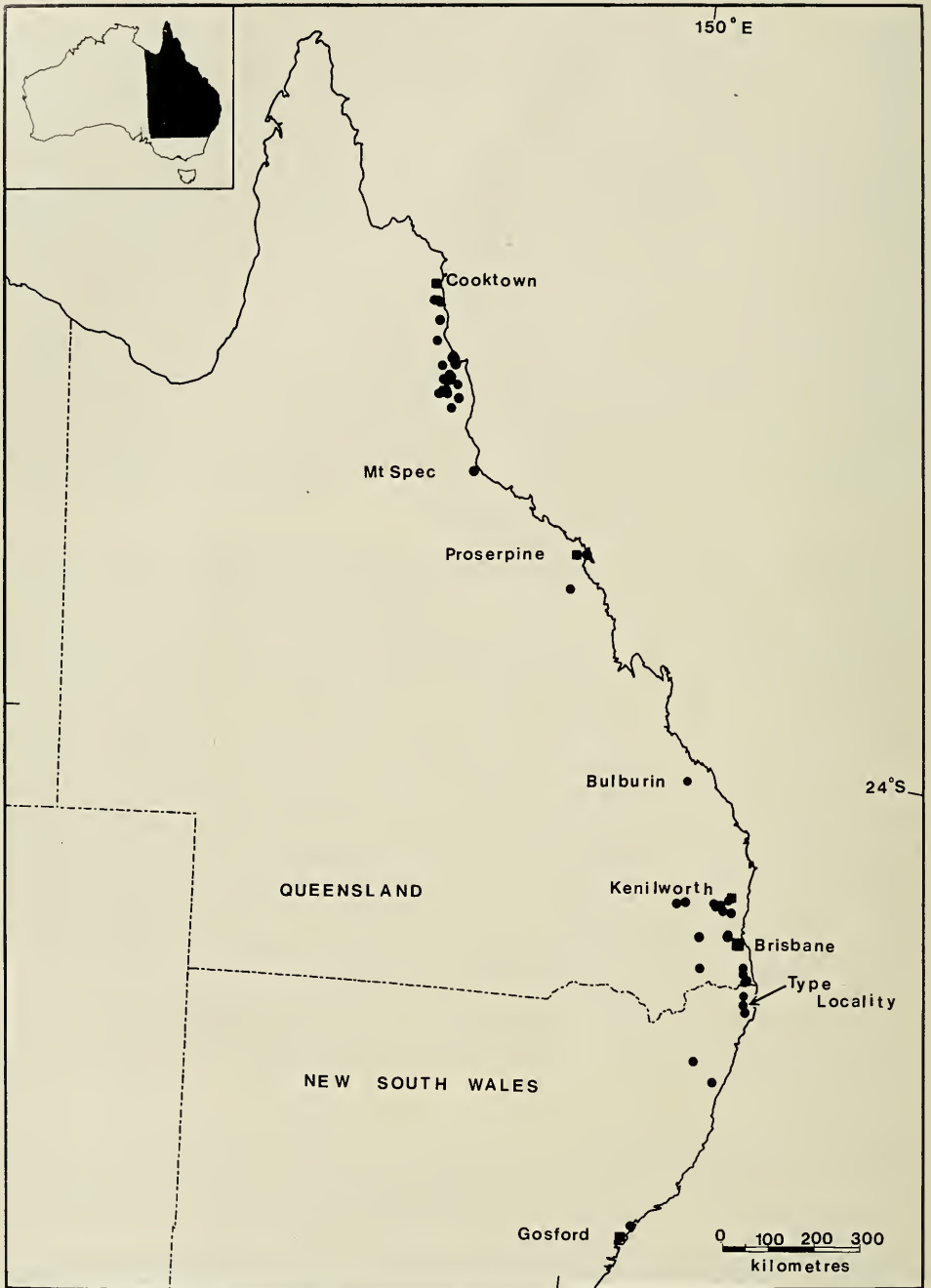


FIG. 1. Distribution of *Litoria chloris* (Boulenger).



3. Southern population; that area ranging from Kenilworth to Gosford.

The three populations will be referred to as northern, central and southern populations throughout this paper.

## RESULTS

Detailed descriptions of representatives of the southern population are followed in text by comparisons with the other two geographical isolates. Morphometric measurements are expressed as mean  $\pm$  standard deviation (in mm).

### EXTERNAL MORPHOLOGY

The head is slightly longer than broad (HL/HW  $1.03 \pm 0.03$ ), its length equivalent to about one third of the snout to vent length (HL/S-V  $0.31 \pm 0.02$ ). The snout is truncate when viewed from above, slightly rounded in profile and is not prominent. The nares are more lateral than superior, their distance from the end of the snout being less than one half that from the eye. The distance between the eye and naris is usually greater than the internarial span (E-N/IN =  $1.07 \pm 0.11$ ). The canthus rostralis is slightly defined and gently rounded. The eye is large and prominent ( $4.75 \pm 1.22$ ), its diameter greater than the eye to naris distance. The tympanum is distinct, the tympanic diameter being slightly less than the eye diameter ( $3.37 \pm 0.39$ ) and separated from the eye by a distance of about  $\frac{2}{3}$  of its own diameter. The vomerine teeth are in two small round groups lying close together on each side of the midline and on a level with the posterior borders of the choanae. The tongue is moderately large and oval with a slightly indented posterior edge.

The fingers are short and narrowly fringed laterally. Their order of length  $3 > 4 > 2 > 1$ . The webbing between the third and fourth fingers reaches the top of the subarticular tubercle at the base of the penultimate phalanx. The terminal discs are prominent.

The hind limbs are moderately long and slender (TL/S-V  $0.53 \pm 0.02$ ). Toes in order of length are  $4 > 3 > 5 > 2 > 1$ . The webbing of all toes except the fourth reaches the base of the discs. On the fourth toe, the webbing extends to a point slightly above the subarticular tubercle at the base of the penultimate phalanx. There is a small oval inner metatarsal tubercle and a scarcely detectable oval outer metatarsal tubercle.

The dorsal and lateral surfaces of the head and body are finely granular. Prominent plicae are present on the posterior surfaces of the forearm and tarsus. There is a prominent and slightly curved supratympanic fold extending from the eye to a point adjacent to the insertion of the forearm. The throat, chest and abdomen are granular and the lower surface of the thighs tubercular.

In the male, the nuptial pad of the first finger is glandular extending to the base of the disc. A submandibular vocal sac is present.

*Colour in preservative:* The dorsal surface of the head, body, forelimbs, fourth finger, tibia, posterior edge of tarsus, and dorsal surfaces of fourth and fifth toes are pale blue. The dorsal and posterior surfaces of the thighs are mauve to deep violet. The plicae on the forearm and tarsus are white.

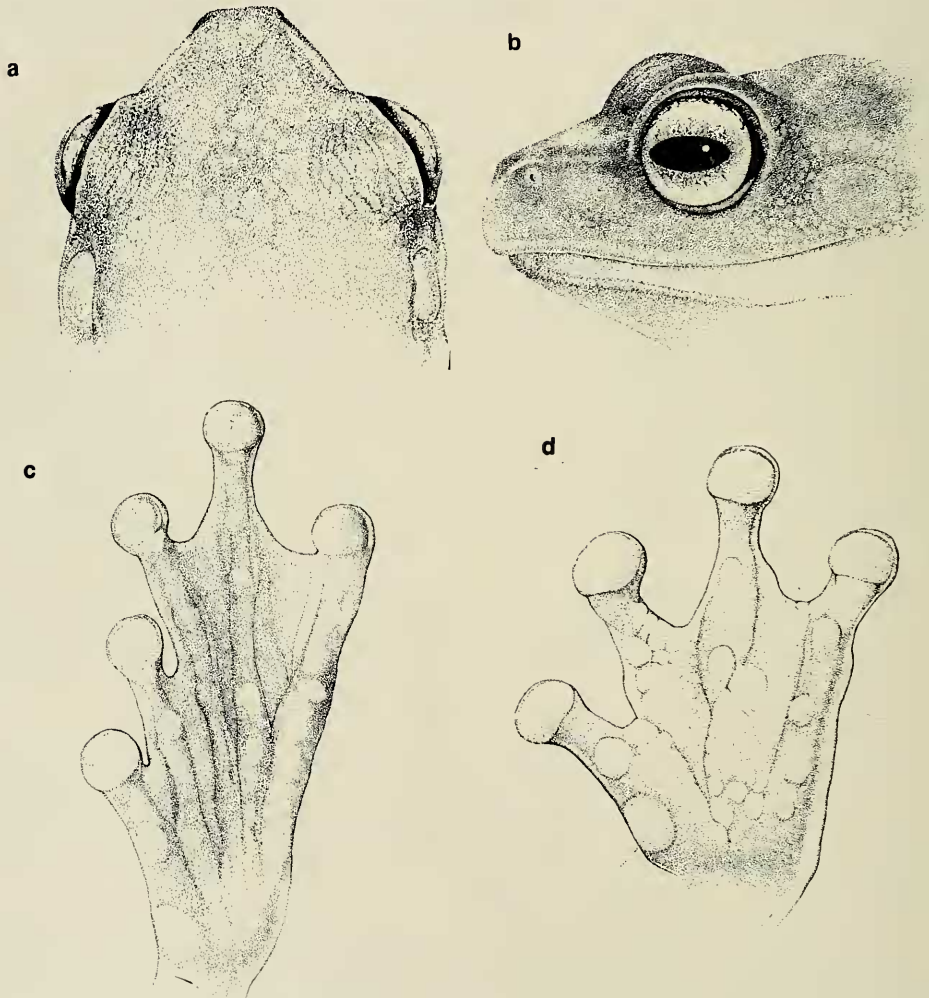


FIG. 2. a) Dorsal and b) lateral view of head, c) foot, and d) hand of *Litoria chloris*, SAM R.16828, from Eungella National Park, Qld (central population).

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*Colour in life:* Those portions of the frog that are pale blue in preservative are a brilliant lime in life. Thigh colour ranges from bluish mauve to purple. The iris is peripherally a deep orange to red with a pale border surrounding the pupil. In males the submandibular region is a brilliant yellow.

### VARIATION BETWEEN THE THREE POPULATIONS

Morphological variation between the three populations is shown in Figure 3.

The head is longer than broad in both the central (HL/HW  $1.03 \pm 0.03$ ) and northern populations (HL/HW  $1.06 \pm 0.03$ ). The head length/head width ratio of the northern population is significantly different from that of the other two populations. The head length/snout-vent lengths in all populations are approximately equal (HL/S-V =  $0.32 \pm 0.02$  in both central and northern populations). Head and snout shape do not vary between frogs from different localities (Fig. 2). In all cases the distance from the eye to naris is greater than the internarial span (E-N/IN =  $1.04 \pm 0.10$  in the central population and  $1.11 \pm 0.15$  in the northern population). E-N/IN in the northern population differs significantly from the control population but not from the southern population. The eye is large and prominent in all specimens ( $4.63 \pm 0.40$  — central, and  $4.95 \pm 0.51$  — northern), its diameter always being greater than the eye to naris distance. The tympanum diameter is less than the eye diameter in all specimens ( $2.99 \pm 0.28$  in central frogs and  $3.05 \pm 0.41$  in northern frogs).

Position of the vomerine teeth does not vary, nor does the general shape of the tongue. Webbing, finger and toe lengths and size of discs do not vary between populations (Fig. 2). There is little to no variation between relative tibia and snout-vent lengths in all three populations (TL/S-V =  $0.54 \pm 0.03$  in both central and northern populations), yet both snout-vent lengths and tibial lengths are significantly different between all three populations. Frogs from the southern population are larger than frogs from the northern one and the latter are larger than those from the central population.

Body colouration varies little between the three populations with the exception of the absence of a narrow green streak running along the dorsal surface of the thighs in frogs from the central population and the considerable variation in thigh colour between the three groups. In comparison with the thighs of southern frogs, the colour of the thighs of central frogs is more intense, being a deep blue in life and purple in preservative whilst the thighs of northern *L. chloris* are bright orange in life, and colourless in preservative. Variation in iris colour is also apparent, being a golden orange in northern frogs as opposed to the more reddish tint of the other two groups.

### CRANIAL OSTEOLOGY

Dorsal, ventral and lateral views of the skull of *Litoria chloris* (U.A.Z. D329 — southern population) are shown in Figure 4. The skull is moderately well

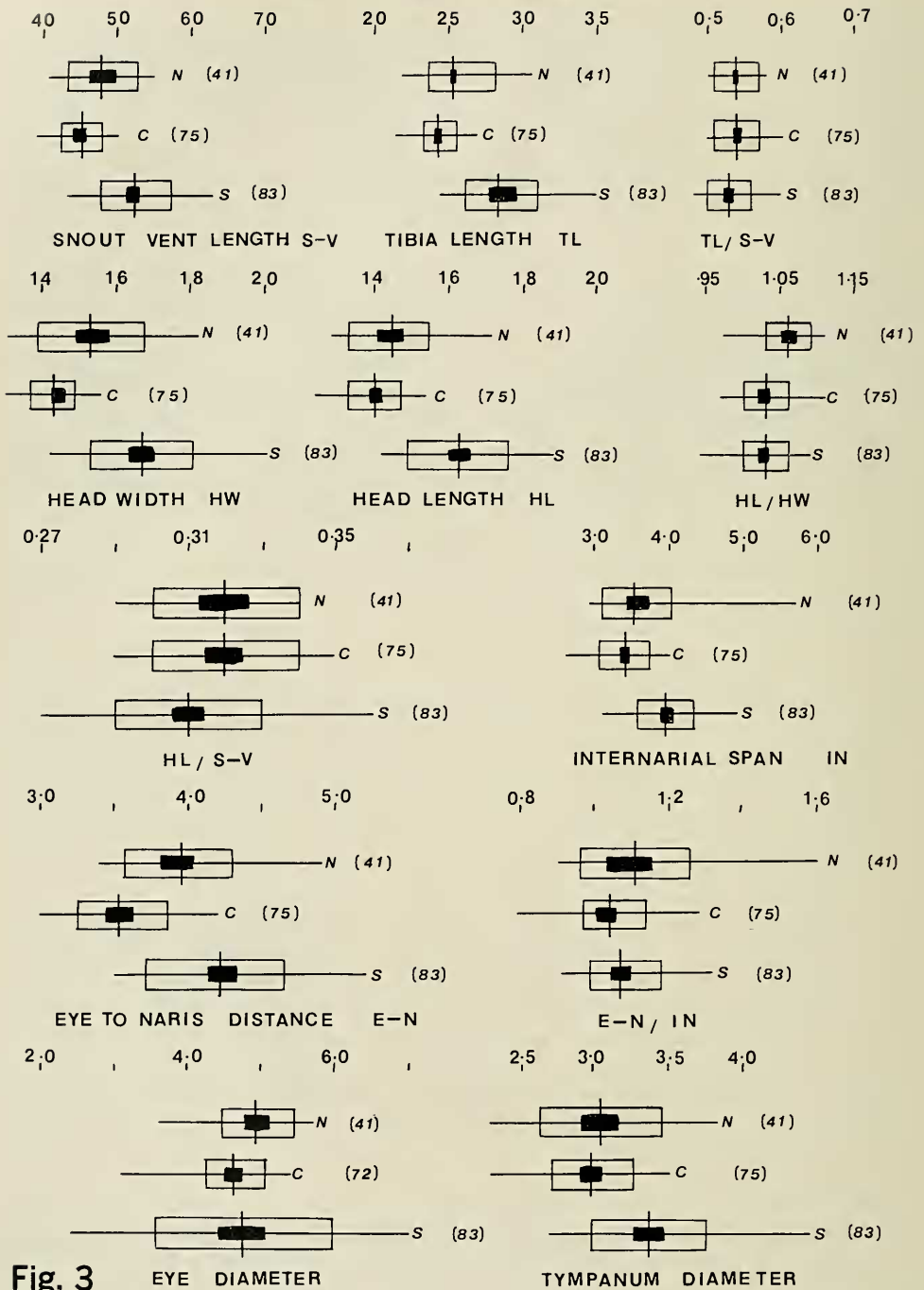


Fig. 3



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ossified and slightly broader than it is long. A large portion of the sphenethmoid is ossified to lie between the nasals dorsally and the prevomers ventrally. The prootic and exoccipital regions are completely fused. The crista parotica are well developed, moderately narrow laterally with prominent epiotic eminences. The otic rami of the squamosals lie alongside the lateral extremities of the crista parotica. The zygomatic ramus of the squamosal is slightly longer than the otic ramus and extends one third of the distance to the maxillary. The frontoparietal fontanelle is moderately sized and is ovoid, the anterior margin being formed by the sphenethmoid about one third posteriorly along the length of the orbit; the posterior margin is approximately level with the posterior edge of the orbit. The orbital edges of the frontoparietals are straight and there are no crests or flanges.

The nasals are moderately narrow bones with acuminate maxillary processes making contact with the preorbital process of the pars facialis of the maxillary. The nasals overlap the sphenethmoid along their posterior edges. The palatines are long and slender medially, not ridged and are expanded laterally to lie alongside the maxillary. The parasphenoid is robust, the cultriform process being acuminate and extending for  $\frac{2}{3}$  of the length of the orbit. The alae are moderately narrow, of moderate length and directed slightly posterolaterally. The pterygoid is well developed, the short medial arm not being in bony contact with the prootic region and the anterior arm stretching for approximately  $\frac{2}{3}$  of the length of the orbit.

The quadratojugal is slender and in firm contact with the maxillary. The squamosals are moderately robust with a slightly expanded otic plate. A bony columella is present. The maxillaries and premaxillaries are dentate. The pars facialis of the maxillary is deep with a moderately developed preorbital process. The alary processes of the premaxillaries are moderately separated medially, expanded dorsally and slightly inclined posteriorly. The palatine processes of the premaxillaries are moderately well developed and meet at their extremities. The palatal shelf is moderately narrow with a moderately developed pterygoid process. The prevomers are entire with short dentigerous processes. The alae of the prevomers form the anteromedial margins of the choanae.

### APPENDICULAR SKELETON

There are eight procoelous, non-imbricate presacral vertebrae. The sacral diapophyses are moderately expanded and the ilia extend anteriorly to them.

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FIG. 3. Morphological variation in three populations of *Litoria chloris*. The short vertical line is the sample mean and the horizontal line is the range of variation. The black bar represents the 95% confidence limits on each side of the mean and the open bar indicates one standard deviation of the mean. The number of observations is indicated in parentheses. A significant difference between samples is indicated by non overlap of the black bars. N = Northern Population; C = Central Population; S = Southern Population.

Sacrococcygeal articulation is bicondylar. The relative lengths of the transverse processes of the presacral vertebrae are: Sacrum > III > IV > V = VI = VII > VIII > II.

The arciferal pectoral girdle is robust and both prezonal and postzonal elements are present. The coracoids are well developed and not closely juxtaposed and the clavicles are slender and strongly arched.

The intercalary structures are not ossified and there is no flange present on the third metacarpal. The phalangeal formulae are 2, 2, 3, 3; 2, 2, 3, 4, 3.

#### VARIATION

Variation in cranial characters between the three populations is minimal but that observed such as the distribution of ossification of the sphenethmoid and nasals may be attributed to ontogenetic effects. The alae of the parasphenoid appeared slightly longer in both central and northern populations than in southern frogs, but quantification is difficult.

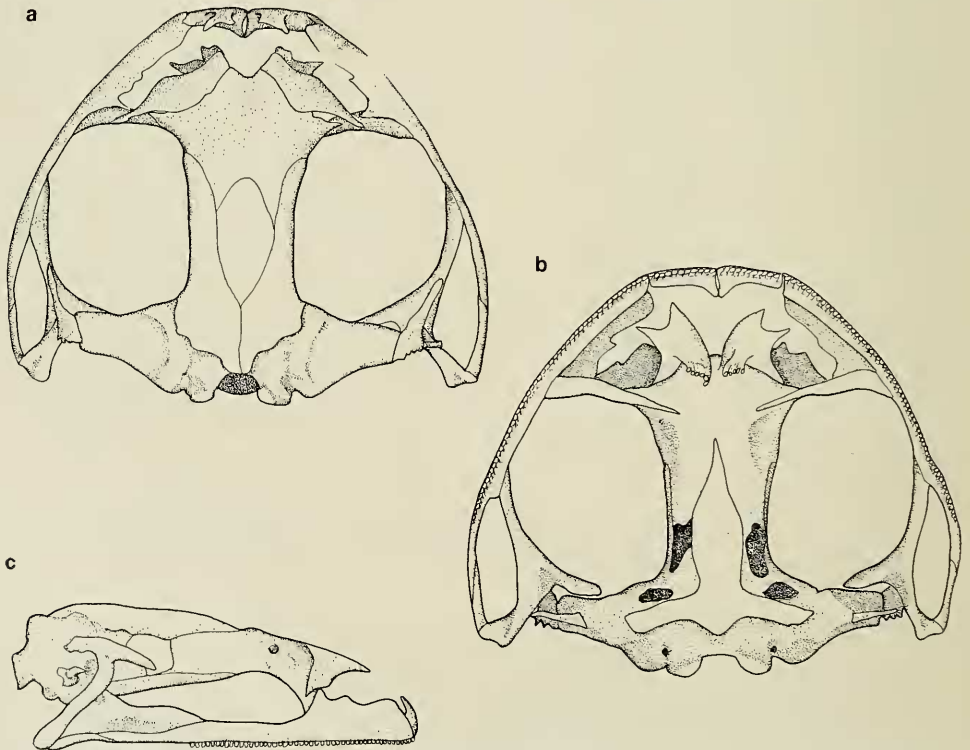


FIG. 4. a) Dorsal, b) ventral, and c) lateral views of the skull of *Litoria chloris* (UAZ D329) — Springbrook National Park, Qld (southern population).

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Variation was apparent in the relative lengths of the transverse processes of the presacral vertebrae. In central frogs III > Sacrum > IV > V = II = VI = VII = VIII whilst in northern frogs III > Sacrum > IV > VI = VII = VIII > V > II.

Sacral diapophyses expansion and relative ilia position together with other appendicular skeletal characters recorded for southern populations showed little or no variation in all groups.

### HABITAT

*Litoria chloris* occurs high in the trees in a wide range of closed forest habitats, at altitudes ranging from 5-950 m above sea level. Frogs have been collected in Araucarian microphyll vine forest, notophyll and mesophyll vine forests and sclerophyll vine forest (nomenclature of Webb, 1959). No preference for a particular forest type is apparent. It is rarely encountered except during the breeding season when individuals descend to the ground and can be found at suitable pools within the forest or in adjacent areas.

### BREEDING BIOLOGY

In Queensland breeding occurs between October and March with a peak of activity from November to February. Spasmodic calling may be heard as early as September if rain falls. Heavy rainfall is a prerequisite for breeding as in *L. gracilentata* and *L. caerulea* (K.R.M., unpublished data).

Large congregations of calling males form around static pools at the edge of streams, and semi-permanent or ephemeral water bodies including road drains, water tanks and flooded quarries. The size of the breeding congregation may be influenced by the rainfall; in the Bunya Mountains, Queensland, in January 1974 more than 220 males were found calling along a 10 x 1 m roadside gutter transect during cyclonic rain.

The call is a growl lasting about 1 second which is repeated many times at increasing intensity. The last growl in a sequence is followed by two or three short trills. We were unable to detect differences by ear in call structures between males of the three populations.

Amplexus is axillary (characteristic of *Litoria*) and occurs near the breeding site; the pair then moves into the water. Spawn is deposited as a clear, flat, jelly-like mass on the surface. In shallow pools spawn has been found covering the surface whereas in pools deeper than 8 cm, it is deposited at the edges. Twelve masses of eggs from the Conondale Range had a mean of 1306 eggs in each, standard deviation of  $\pm 228$  and range 812-1561. Eggs have dark brown animal poles and separate jelly capsules. Fertile eggs sink and adhere to twigs, leaves or grass. Watson and Martin (in press) describe larval structure and life history.

Other species sharing *Litoria chloris* breeding sites include *L. gracilentata* (Peters), *L. lesueuri* (Duméril and Bibron), *L. pearsoniana* (Copland), *Lechriodius fletcheri* (Boulenger), *Adelotus brevis* (Gunther), *Mixophyes fasciolatus* Gunther, *M. iteratus* Straughan, *Rheobatrachus silus* Liem and *Taudactylus diurnus* Straughan and Lee.

## DISCUSSION

The external morphology and osteology of the three geographically separated populations of *Litoria chloris* were analysed. Little or no variation in osteology was detected and variation in external morphology is limited to colour pattern and head shape. While the colour of the thighs in the central population is closely similar to that of the southern population, individuals from the north are markedly different being orange instead of deep blue or mauve to purple. The northern population further differs from the central and southern populations in the head length/head width ratio but these rather minor differences do not warrant recognising the northern population as a distinct taxon. Straughan (1966) was also unable to differentiate between the call structure of the northern and southern populations.

Faunistically each of the areas is characterized by the existence of a number of endemic species of amphibians and lizards. In the northern area, *Taudactylus acutirostris* (Anderson), *T. rheophilus* Liem and Hosmer, *Litoria nannotis* (Andersson), *Goniocephalus boydii* (Macleay), *Sphenomorphus tigrinum* (de Vis) and *Carphodactylus laevis* Gunther occur, and in the central area there are *T. eungellensis* Liem, *Phyllurus caudiannulatus* Covacevich and two undescribed *Sphenomorphus* species. Endemic species found in the southern area are *Lechriodius fletcheri* (Boulenger), *Egernia major* (Gray), *Goniocephalus spinipes* (Duméril) and *Sphenomorphus murrayi* (Boulenger).

At present it is premature to speculate about the causal factors of this high degree of speciation but, in so far as the present study is concerned, it is conceivable that the populations of *Litoria chloris* analysed here have been subjected to the same pressures, and are in the process of differentiating. However, they may have attained already unique biological integrity.

In the past, the degree of morphological variation reported here has been considered sufficient to merit the erection of new species in populations studied in the southeast and southwest of Australia (e.g. Copland 1957). However, in the absence of supporting biological data such as mating calls and comparative larval morphology, we consider it would be premature at this stage to recognise separate taxa.



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